

FIG. A -- Compound 15 increases MCIP1 protein expression. Western blot with anti-MCIP1 antibody. Protein prepared from neonatal rat ventricular myocytes exposed to vehicle (DMSO) or compound 15 (10 μM) for 48 hours.

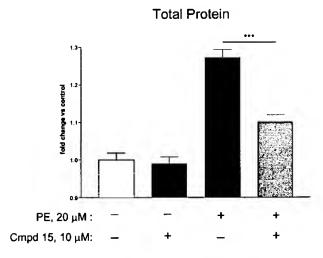


FIG. B — Compound 15 attenuates phenylephrine-induced increases in total protein. Total protein was measured in neonatal ventricular myocytes exposed to the hypertrophic agonist phenylephrine and compound 15 for a period of 48 hours.

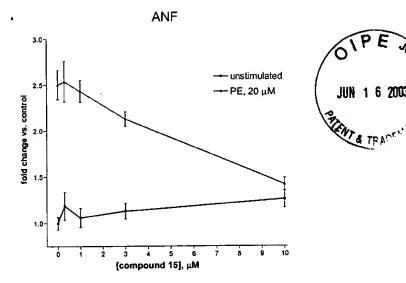
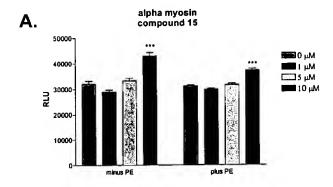


FIG. C -- Compound 15 attenuates phenylephrine-induced increases in secreted ANF. Secreted ANF was measured in neonatal ventricular myocytes exposed to the hypertrophic agonist phenylephrine and compound 15 for a period of 48 hours



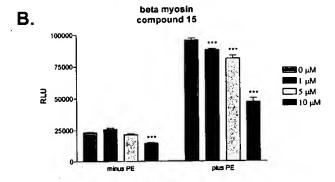


FIG. D -- Compound 15 reverses hypertrophic changes in myosin heavy chain isoform expression in cardiac myocytes. A) Alpha myosin heavy chain expression in neonatal rat ventricular myocytes in the presence or absence of phenylephrine and treated with three concentrations of compound 15. B) Beta myosin heavy chain expression.

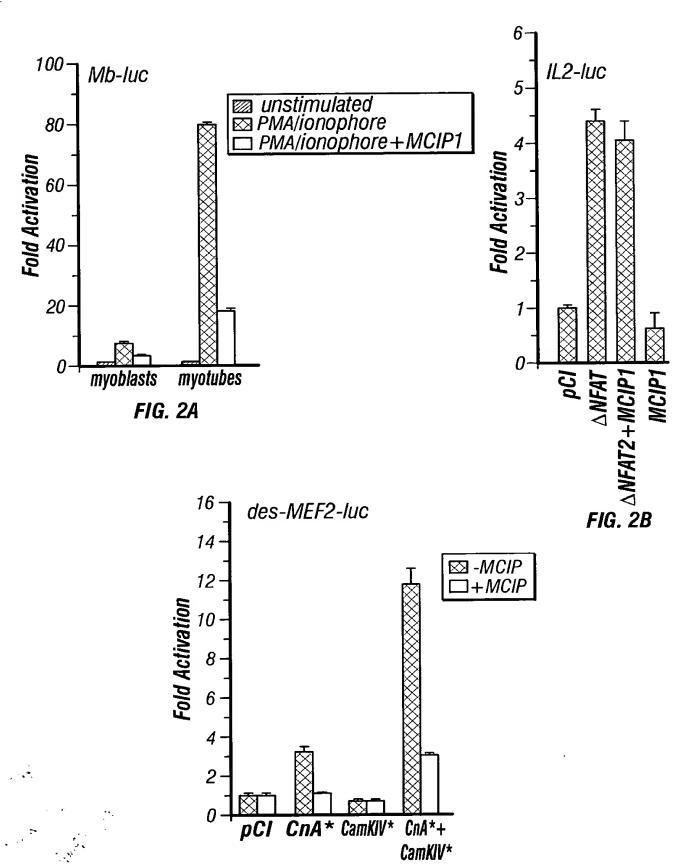


FIG. 2C

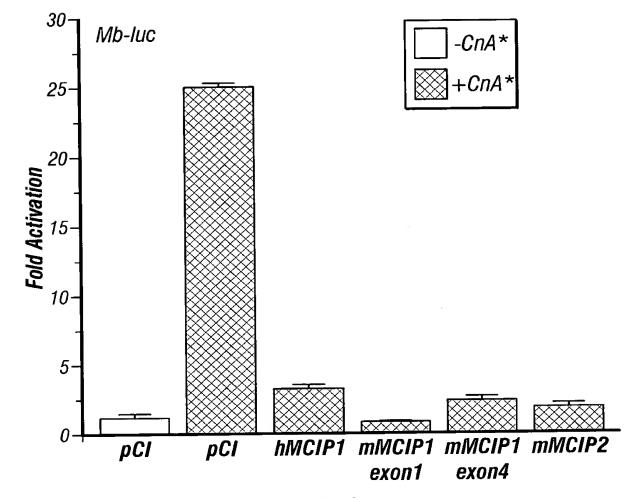


FIG. 3



MCIP1 Binding	Calcineurin A protein	521 aa			
	catalytic domain B M				
++++					
++++	342 aa				
-					

FIG. 4A

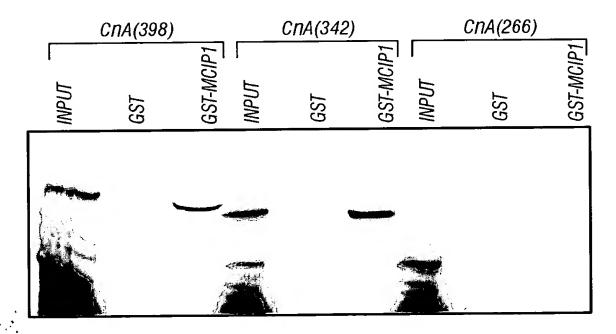


FIG. 4B



## U.S. Serial No. 09/782,953 METHOL OD COMPOSITIONS RELATING TO MUSCLE SELECTIVE CALCINEURIN INTERACTING PROTEIN (MCIP) Inventor(s): Williams et al.

<u>CnA Binding</u> ++++	MCIP1 protein	197 aa ]
++		
++		
+		

FIG. 5A

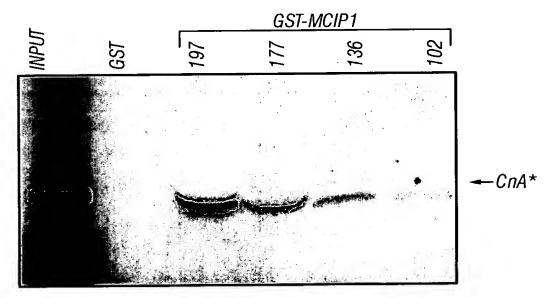
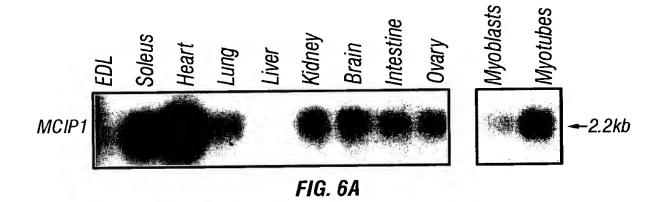


FIG. 5B



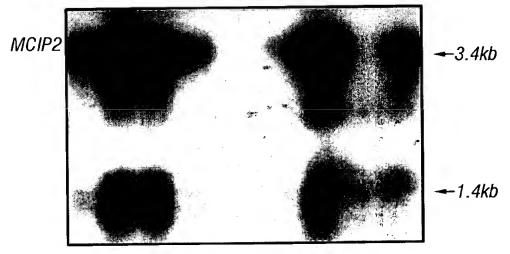


FIG. 6B

Rank	Fold	Gene	Genbank ID						
hypertrophic α-MHC-CnA* vs. wild-type									
1	8.1	Calcineurin-A	AA245461						
2	4.0	ANF precursor type B	AA030805						
3	3.3	ANF precursor type A	W14325						
4	3.1	sk mus LIM protein (FHL1)	AA047966						
5	3.0	OSF-2	W81878						
<b>→</b> 6	2.7	MCIP-1	AA200984						
7	2.7	EST (mouse)	AA110791						
8	2.3	MCPSF (Mouse cleavage	AA221269						
		and polyadenylation factor)							
failing α-MHC-CnA* vs. hypertophic α-MHC-CnA*									
1	3.3	Procollagen XV	W83331						
2	2.9	OSF-2	W81878						
3	2.8	EST (mouse)	AA124355						
4	2.7	Alphà-crystallin	AA231358						
<b>→</b> 5	2.5	MCIP-1	AA200984						
6	2.2	Procollagen III	W89883						
7	2.1	p53BP2	AA467287						
8	2.1	Calcineurin-A	AA245461						

FIG. 7



	AATGGGTTAA	AATGATCGAA	TTICAAATGA	CTGTGGGATA	GGGAAAAAA	AAAATTTTA	AAAGTGAAAC	TCACCCGGAC	AGTCTAATTG	CAITTTCAI	CTGAGTAGCT	GTTGATAAAG	CAAGCAAAGT	TCTTTCCAGI	CGACTTGACT	CTTTTTTT	CCAGCTCAAG	
2kb 3.5kb E7		GTGCTTTGAA	ATAATGAAGT	CTCCATTCCA	AATGTCACTG	TTTGTCCTTT	CTAATACCCC	ATCAAGTATG	ATTTCCCTAC	TCTTTTGTAA	AGCGTGTTGT	TGCAAGAGAG	CCATICICCC	TGACGTCAC	CAAGCATCCA	GGAAGCTGCA	TTTATAAGCA	
3340	CAAC	GAATTCTGTA	TCAAATTATA	GTTCTGTTTG	CATCCAATAA	CATTCCTCTT	AATGTTTTCT	AATTCCAGGG	AAGTCTTGAA	TCATCCTTTT	TCGGCCGAGG	GAAACTATGC	GCTGITTITT	ACTAAGGTGT	TGTCTACCTG	AGGATGCTGT	CCCTTACTGC	GAAAGGAAT
-35kb	6 E E C 6 C	CICAGITICA	TCCCCAGTTC	ATTGTTTTAG	TTGGGACATT	ACTTCAAAAT	CAGATTTCAA	GTTTTCAGGA	AGGTTTCCCA	CCACAGACCT	GICGICCCAI	ACTACGAGTG	GCAAACCTCA	AGGGAAAATG	ACTTTGTCCC	CTGTCCAAAC	TGGGGGCTGG	CAGOCTCTTG
	C E E C C E E C C E E C C E E C C E E C E E C E E C E E E C E	IGUTTOTIG	GTGTTTCATA	AAATOCTAGG	CAAGTAGAAA	TTTTAACIAC	TTGACCAAGA	TTTGATTGAG	TTIGGTTTCC	CTGTTTAT	CTTAAGAAGG	GAATGGAATT	CAGCTGTCAA	TAATTAGOAT	<b>AGAAACTT</b> AC	CAGGAATTTG	CCCCAGGGAG	AAGGAACCTA
	-874	0081	-750	-700	-650	009-	-550	-500	-450	-400	-350	-300	-250	-200	-150	-100	-50	+1

FIG. 8A



MCIP exon 4 promoter constructs
$$-874 +30$$

$$(15) NFAT SITES LUC$$

$$-231 +30$$

$$(5) LUC$$

$$-168 +30$$

$$(3) LUC$$



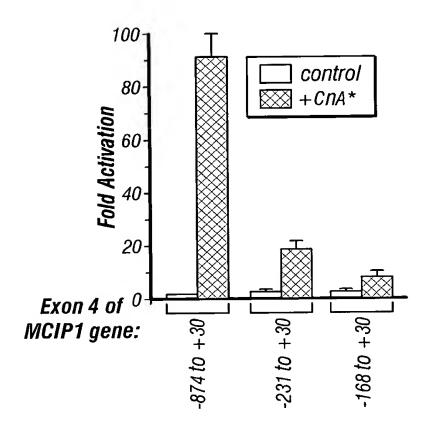


FIG. 8C



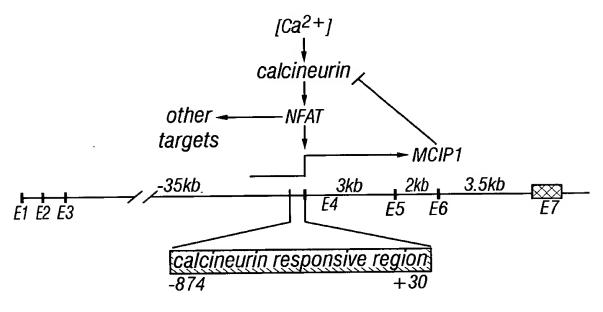


FIG. 9

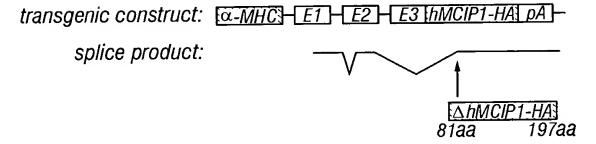
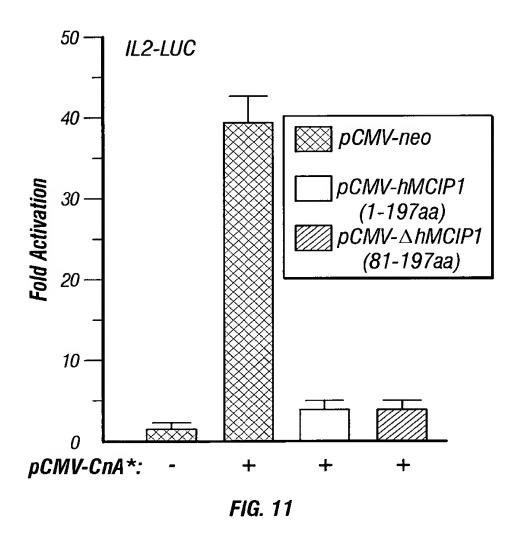


FIG. 10



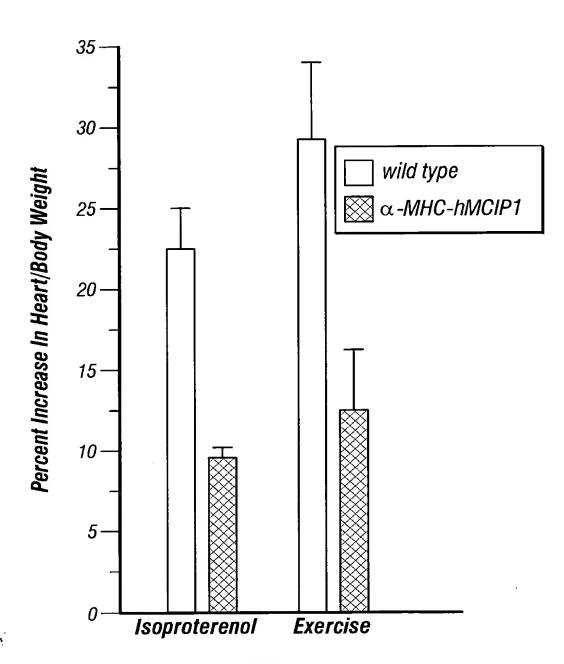


FIG. 12